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## Selenoprotein expression in endothelial cells from different human vasculature and species

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### Abstract

Selenium (Se) can protect endothelial cells (EC) from oxidative damage by altering the expression of selenoproteins with antioxidant function such as cytoplasmic glutathione peroxidase (cyGPX), phospholipid hydroperoxide glutathione peroxidase (PHGPX) and thioredoxin reductase (TR). If the role of Se on EC function is to be studied, it is essential that a model system be chosen which reflects selenoprotein expression in human EC derived from vessels prone to developing atheroma. We have used [<sup>75</sup>Se]-selenite labelling and selenoenzyme measurements to compare the selenoproteins expressed by cultures of EC isolated from different human vasculature with EC isolated from bovine and porcine aorta. Only small differences were observed in selenoprotein expression and activity in EC originating from human coronary artery, human umbilical vein (HUVEC), human umbilical artery and the human EC line EAhy926. The selenoprotein profile in HUVEC was consistent over eight passages and HUVEC isolated from four cords also showed little variability. In contrast, EC isolated from pig and bovine aorta showed marked differences in selenoprotein expression when compared to human cells. This study firmly establishes the suitability and consistency of using HUVEC (and possibly the human cell line EAhy926) as a model to study the effects of Se on EC function in relation to atheroma development in the coronary artery. Bovine or porcine EC appear to be an inappropriate model.

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### 1. Introduction

The intake of selenium (Se) can to be inversely correlated with the incidence of atherosclerosis and coronary heart disease [1,2]. Oxidative damage to the endothelium is thought to be a primary event in the pathogenesis of atherosclerosis and Se, added as selenite, can protect the EC from such damage by altering the expression of specific selenoproteins [3,4]. The intracellular selenoproteins identified to date as having a potential antioxidant function include cytoplasmic glutathione peroxidase (cyGPX), phospholipid hydroperoxide glutathione peroxidase (PHGPX) and isoenzymes of thioredoxin reductase (TR).

Labelling cells with [<sup>75</sup>Se]-selenite provides a sensitive method for assessing the expression of selenoproteins. Since

equilibration of exogenous [<sup>75</sup>Se]-selenite with the endogenous pool of Se and selenoproteins can take in excess of 24 h [5,6], such labelling experiments require incubation with [<sup>75</sup>Se]-selenite for 36–48 h. Using these techniques to detect selenoproteins in human umbilical vein endothelial cells (HUVEC), TR has been identified as the predominant selenoprotein comprising 43% of the total selenoproteins [6]. Both cyGPX and PHGPX are also expressed by cultured EC obtained from a number of species including humans [4,7,8] but the relative activities of TR in EC isolated from different species have not been defined.

The culture of EC derived from the large vasculature is a well-established model for the study of the endothelium. Ideally, EC isolated from human coronary arteries would be preferred for studies relating to cardiovascular disease in man since atheroma is common in these vessels and is a major cause of morbidity and mortality. In practice, the human umbilical vein is often the chosen source of EC for the study of human endothelial function because of its accessibility.

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Moreover, it is a non-branching vessel with a large intimal surface area, making it technically easy to isolate cells. Unfortunately, the viability of isolated EC can be modified by several factors including foetal stress, maternal anaesthesia, smoking and other toxins [9,10]. The use of HUVEC and other primary cultures of EC is also complicated by genetic variability between preparations, limited population doublings and the requirement for specialised growth factors. Arterial and venous EC show differences in the production of angiotensin-converting enzyme [11] and their response to cytokine stimulation [12]. These observations have led to the suggestion that HUVEC, despite being widely used by researchers in the field of vascular disease, may not be the most suitable model for studying human cardiovascular disease [13–15].

Bovine aortic endothelial cells (BAEC) and porcine aortic endothelial cells (PAEC) are also often used as models to study EC function. PAEC may be a suitable alternative to HUVEC since there are similarities between the porcine and human cardiovascular system [16]. In addition, porcine aorta is subject to atheroma formation and has been used as a model for the study of this process [17]. However, variation between EC isolated from different species has been acknowledged to occur; for example, PAEC, unlike HUVEC and BAEC, do not express Factor VIII-related antigen [17].

The EC line EAhy926, established by hybridising primary HUVEC with A549 human lung tumour cells [18], has been used in a number of studies of EC function. EAhy926 retain many of the differentiated functions common to primary EC beyond 100 passages. These functions include: the expression of von Willebrand Factor [18]; prostacyclin formation [19]; expression of endothelin-1 [20]. The selenoprotein profile of EAhy926 cells has not been previously determined, but such work is essential in order to establish whether this convenient cell line would provide a suitable model for future studies of selenoprotein expression in EC.

If the role of Se on EC function is to be studied, it is essential that a model system be chosen which reflects the selenoprotein expression and function of human EC derived from vessels prone to developing atheroma. The experiments reported here use [ $^{75}\text{Se}$ ]-selenite labelling and enzyme measurements to compare the selenoproteins expressed by cultures of EC isolated from different human vasculature with EC isolated from bovine and porcine aorta. The selenoprotein profile of the human EC cell line EAhy926 has also been studied.

## 2. Methods

### 2.1. Isolation and culture of EC

#### 2.1.1. HUVEC and human umbilical artery endothelial cells (HUAEC)

Human umbilical cords (>100 mm in length) were obtained at normal deliveries or Caesarean section from

nonsmoking women. Immediately after delivery, the cords were placed into sterile Earle's balanced salt solution (EBSS) containing penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml) and kept at 4 °C. EC were isolated within 20 h of delivery using a method adapted from that described previously by Anema et al. [6] and Jaffe et al. [21]. Cells were cultured in Endothelial Growth Medium-2 (EGM-2: Biowhittaker, Berkshire, UK) containing penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml) at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air.

The cells showed the morphology characteristic of EC in culture previously described by Jaffe et al. [21]. Cells also synthesised von Willebrand Factor as determined by an indirect immuno-fluorescent detection system.

#### 2.1.2. Human coronary arterial endothelial cells (HCAEC)

These were purchased from Biowhittaker, and tested positive for the presence of von Willebrand Factor and acetylated LDL (an alternative method for the specific characterisation of EC in culture) [22]. The HCAEC also displayed the characteristic morphology of EC. HCAEC were maintained in EGM-2 containing penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml), and cultured at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air.

#### 2.1.3. EAhy926 EC line

EAhy926 cells were kindly donated by Professor Cora-Jean Edgell of the University of North Carolina, North Carolina, USA. The cells were maintained in high glucose (4.5 g/l) Dulbecco's Modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS), 5 mM hypoxanthine, 0.02 mM aminopterin, 0.8 mM thymidine, penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml). The cells were incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air and were passaged weekly using 0.25% trypsin–0.02% EDTA solution. EAhy926 cells displayed the characteristic morphology of EC in culture and stained positive for vWF.

#### 2.1.4. BAEC

These were purchased from Biowhittaker. The certificate of analysis supplied with this product stated that the BAEC tested positive for acetylated LDL. The cells were maintained in EGM (Biowhittaker) containing penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml) and incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air.

#### 2.1.5. PAEC

Porcine aorta was obtained within 5–10 min of slaughter from pigs aged under 2 years and was immediately placed into sterile EBSS at 4 °C containing penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml). EC were isolated within 2–3 h of dissection using a method adapted from that previously described by Slater and Sloan [16]. Briefly, segments of about 5–10 cm were cut and any

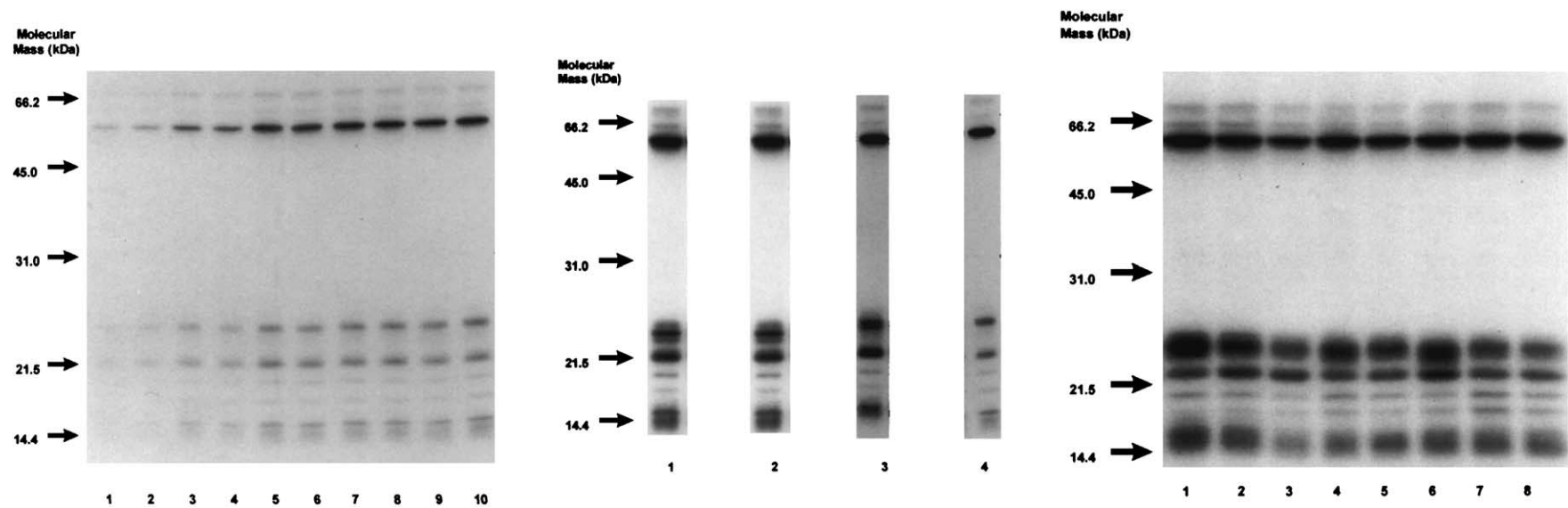


Fig. 1. Left. Autoradiograph of an SDS-PAGE gel of the intracellular selenoproteins of HUVEC labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for various lengths of time over a 96-h period. Duplicate flasks of HUVEC were labelled for each time point. Lanes 1, 2: 12 h; lanes 3, 4: 24 h; lanes 5, 6: 48 h; lanes 7, 8: 72 h; lanes 9, 10: 96 h. Each lane was loaded with 25- $\mu\text{g}$  protein. Middle. Autoradiographs of four SDS-PAGE gels showing the intracellular selenoproteins from four different preparations of HUVEC labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. Each lane was loaded with 25  $\mu\text{g}$  of protein. Right. Autoradiograph of an SDS-PAGE gel of a single isolation of HUVEC at different passages each labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. Lanes 1 to 8 represent passages 1 to 8, respectively. Each lane was loaded with 25  $\mu\text{g}$  of protein.

minor vessels were ligated. The segments were washed with approximately 25 ml of EBSS (prewarmed to 37 °C). One end of the aorta was then clamped shut and the opposite end infused with 0.1% collagenase in EBSS (approximately 10 ml). This end was then closed and the cord incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air. After 15 min the segment was gently massaged and the contents collected into a sterile universal container. The vessel was then cut along its longitudinal axis and the luminal surface gently scraped gently with a sterile stainless steel scalpel blade angled at approximately 60° to the intimal surface. The blade was washed with EBSS and the washings added to the cell suspension previously collected. The sample was then centrifuged at 450 × *g* for 10 min and the resulting cell pellet was washed with the culture medium [M199 containing 20% FBS, penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml)]. The cells were resuspended into a 25-cm<sup>2</sup> flask and incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub>, 95% air. The growth medium was changed and replaced after 24 h and then on alternate days. Cells displayed the characteristic morphology of EC.

## 2.2. Time-course of [<sup>75</sup>Se]-labelling in HUVEC

HUVEC were isolated and maintained as described previously. For labelling with <sup>75</sup>Se-selenite, cells were grown to confluence and the culture medium was changed and replaced with fresh medium containing [<sup>75</sup>Se]-selenite (0.02 MBq/ml). At time 0, 12, 24, 48, 72 and 96 h, the medium was removed from all the flasks and the cells were washed three times with EBSS (4 °C). The cells were harvested into 20 ml of EBSS by scraping and centrifuged at 2000 × *g* for 10 min at 4 °C. The supernatant was aspirated and the cell pellet resuspended in 200 µl of 60 mM Tris buffer, pH 7.8, containing 1 mM EDTA and 1 mM dithiothreitol (Tris buffer). The cells were then lysed by sonication whilst kept at 4 °C on ice.

Protein concentrations were measured using the Bradford assay [23] and the samples were diluted to a common protein concentration with Tris buffer. The cell lysates were subjected to SDS-polyacrylamide gel electrophoresis to separate the [<sup>75</sup>Se]-labelled proteins present in 25 µg of protein. The resulting gel was dried and the [<sup>75</sup>Se]-labelled selenoproteins were visualised by autoradiography using Kodak X-OMAT XAR-5 film.

## 2.3. The effect of passage number on [<sup>75</sup>Se]-labelling in HUVEC

The intracellular [<sup>75</sup>Se]-selenoprotein profiles of eight passages of a preparation of HUVEC isolated from a single umbilical vein were compared. HUVEC were isolated and maintained as described above. The HUVEC from the primary isolate (passage zero) were grown to confluence and passaged into at least three T75 flasks (passage one). At

confluence, HUVEC in two of the T75 flasks were labelled with [<sup>75</sup>Se]-selenite (0.02 MBq/ml) for 48 h, whilst the third was subcultured further to provide passage two. This procedure was continued until cells had reached passage eight, at which point distinct morphological changes were observed, such as significant cell enlargement and a partial loss of the characteristic cobblestone appearance.

After a 48-h labelling period, cells from each passage were harvested and stored at −20 °C. Samples were thawed, lysed and prepared for separation using SDS-PAGE as described above.

## 2.4. The [<sup>75</sup>Se]-labelling of vascular EC isolated from different vasculature

The intracellular selenoprotein profile of HUVEC, HCAEC, HUAEC, EAhy926, BAEC and PAEC were compared. Each cell type was isolated and maintained as previously described above and labelled for 48 h with [<sup>75</sup>Se]-selenite (0.02 MBq/ml) prior to SDS-PAGE electrophoresis. For HUVEC, the selenoprotein profiles in

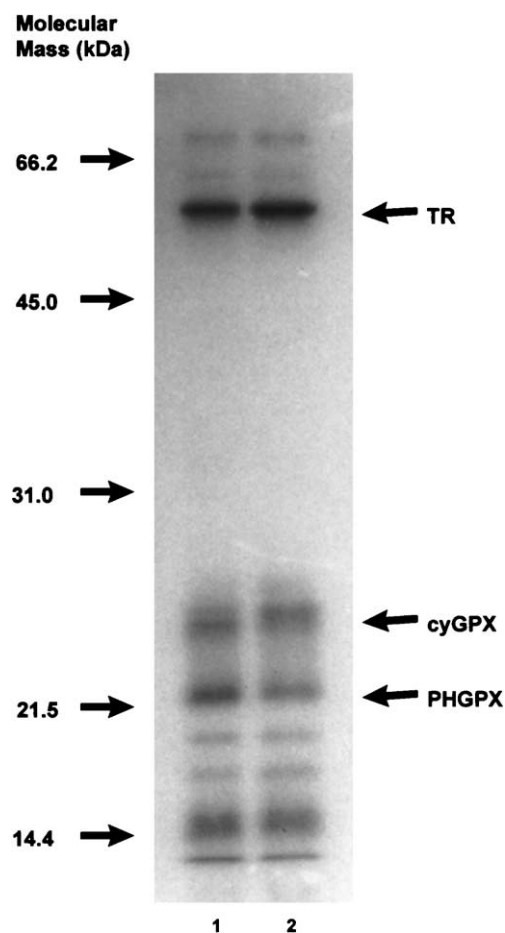


Fig. 2. Autoradiograph of an SDS-PAGE gel of the intracellular selenoproteins of HUVEC and HCAEC labelled with [<sup>75</sup>Se]-selenite (0.02 MBq/ml) for 48 h. Lane 1, HUVEC; lane 2, HCAEC. Each lane was loaded with 25-µg protein. The selenoprotein bands representing cyGPX, PHGPX and TR are indicated.

four different preparations of cells isolated from four different cords were also compared.

### 2.5. Glutathione peroxidase and TR in EC

For each cell type the activities of TR, cGPX and PHGPX were measured in parallel subcultures of EC grown in triplicate 75-cm<sup>2</sup> flasks using the same culture medium as was used for the <sup>75</sup>Se labelling experiments. The activity of the selenoenzymes in EAhy926 cells was measured in cells grown in the DMEM culture medium specified above in the presence or absence of 40 nM selenite. After culture, cells were harvested by scrapping and lysed in 0.125 M potassium phosphate buffer (pH 7.4) by sonication on ice. TR concentration was measured by an in-house radioimmunoassay using antiserum raised against human placental TR. The activity of TR was determined using 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) as substrate in the presence and absence of 720 nM gold thioglucose [24]. The activities of cyGPX and PHGPX activity were determined as described previously [25].

### 2.6. Se content of culture medium

The Se content of the culture medium used for each cell type was determined using a fluorometric assay as described by Olsen et al. [26].

### 2.7. Statistical analysis

An unpaired Student's *t* test (with Welch correction when appropriate) was used to test for significant differences between the levels of TR, cyGPX and PHGPX.

## 3. Results

### 3.1. [<sup>75</sup>Se]-labelling in HUVEC

Fig. 1 (left panel) shows the autoradiograph of a SDS-PAGE gel demonstrating the changes in [<sup>75</sup>Se]-labelling

over time. Selenoproteins were only faintly labelled after a 12-h exposure to [<sup>75</sup>Se]-selenite. The intensity of labelling of all selenoproteins increased up until 48 h, at which time a steady state of labelling was achieved. Three major [<sup>75</sup>Se]-labelled selenoproteins were observed with mean molecular masses of 58.1, 21.7 and 24.4 kDa. A selenoprotein of approximately 15 kDa was also moderately expressed. A number of minor labelled selenoproteins were observed.

No distinct variations in the pattern of intracellular [<sup>75</sup>Se]-labelled selenoproteins between four different preparations of HUVEC were observed (Fig. 1, centre panel) and similarly the selenoprotein profile did not change over eight passages in HUVEC obtained from a single cord (Fig. 1, right panel).

Using antiserum to rat TR and human TR, we have previously identified by Western blotting the 58.1-kDa [<sup>75</sup>Se]-labelled band in HUVEC as TR. Western blotting with antiserum to rat testis PHGPX demonstrated an immunoreactive band that co-migrated with the 21.7-kDa [<sup>75</sup>Se]-labelled band. The same approach using antiserum to human cyGPX failed to visualise an immunoreactive band in HUVEC. However, purified human cyGPX (Sigma Chemical Co., Poole, UK) co-migrated with an identical Rf to the 24.4-kDa protein [<sup>75</sup>Se]-labelled band on the SDS-PAGE gel. The 58-, 21.7- and 24.4-kDa <sup>75</sup>Se-selenoproteins observed on the autoradiograph of the SDS-PAGE gels were thus considered to represent TR, PHGPX and cyGPX, respectively.

### 3.2. Selenoproteins expressed by human EC and the cell line EAhy926

The pattern of [<sup>75</sup>Se]-labelled selenoproteins found in HCAEC and HUVEC were almost identical (Fig. 2). TR appeared to be expressed to a higher degree in HCAEC than in HUVEC and mass measurements (Table 1) confirmed this. No significant difference was observed in the activities of cyGPX or PHGPX between HCAEC and HUVEC (Table 1).

The [<sup>75</sup>Se]-selenoprotein expression in venous EC and arterial EC isolated from the same umbilical cord differed only slightly. In HUVEC an additional selenoprotein with a molecular mass of approximately 27 kDa was labelled,

Table 1  
Selenoproteins expressed by EC and the EC line EAhy926

	HUVEC	HCAEC	EAhy926	BAEC
CyGPX activity (mU/mg protein)	86 ± 0.7	88 ± 0.03	23 ± 0.5** <i>158 ± 24.5*</i>	115 ± 7.0
PHGPX activity (mU/mg protein)	7.15 ± 2.09	8.36 ± 0.01	4.41 ± 1.86 <i>12.5 ± 3.0</i>	4.54 ± 0.76
TR concentration (ng/mg protein)	721 ± 63	1245 ± 335*	871 ± 149 <i>1002 ± 267</i>	ND
TR activity (mU/mg protein)	8.9 ± 0.60	24.4 ± 0.34**	11.7 ± 1.37	1.06 ± 0.16**

Results for EAhy926 are given for cells grown in basal culture medium and results in italics are from cells grown in basal medium supplemented with 40 nM sodium selenite.

TR was not detectable (ND) in BAEC because of the lack of cross-reactivity of the antisera with bovine TR.

\* Results significantly different from HUVEC, *P* < 0.05.

\*\* Results significantly different from HUVEC, *P* < 0.001.



which was not observed in HUAEC. TR expression in HUAEC also appeared to be slightly greater than in HUVEC (Fig. 3).

The pattern of selenoprotein expression in EAhy926 cells closely resembled that observed in HUVEC (Fig. 4). The levels of TR and PHGPX in HUVEC were not significantly different to the levels found in EAhy926 cells grown in the basal medium or when supplemented with 40 nM selenite (Table 1). The activity of cyGPX in EAhy926 grown in basal medium was significantly lower than that found in HUVEC, however, EAhy926 cells supplemented with 40 nM selenite had significantly higher activities of cyGPX than HUVEC (Table 1). Supplementation with 40 nM selenite significantly ( $P < 0.05$ ) increased the expression of TR, cyGPX and PHGPX in EAhy926 cells.

### 3.3. Selenoprotein expression in BAEC and PAEC

Compared to HUVEC, PAEC showed quite distinct differences in the pattern of [ $^{75}\text{Se}$ ]-labelled selenoproteins (Fig. 5, left panel). TR was not the most prominently labelled selenoprotein in PAEC but rather a selenoprotein of 15 kDa.

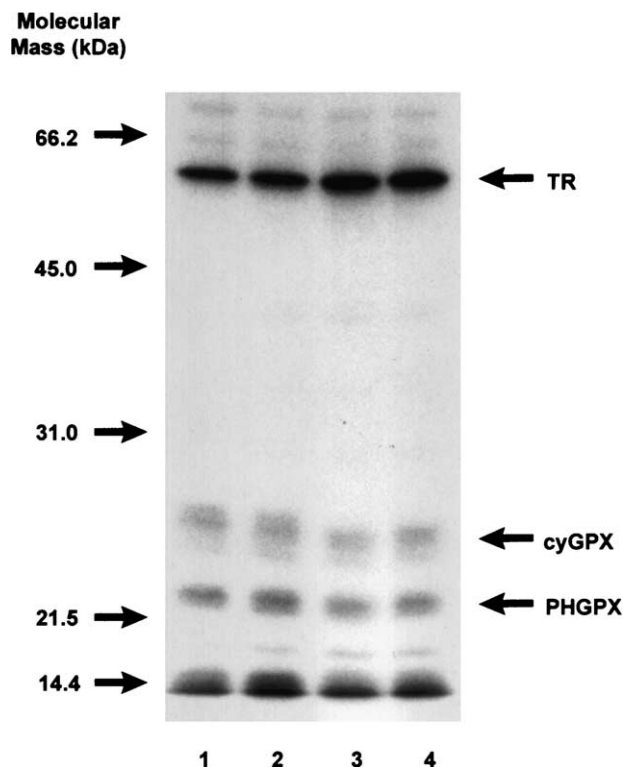


Fig. 3. Autoradiograph of an SDS-PAGE gel of the intracellular selenoproteins of HUVEC and HUAEC labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. Both HUVEC and HUAEC were isolated from a single umbilical cord and samples were taken from duplicate flasks. Lanes 1 and 2, HUVEC; lanes 3 and 4, HUAEC. Each lane was loaded with 25- $\mu\text{g}$  protein. The selenoprotein bands representing cyGPX, PHGPX and TR are indicated.

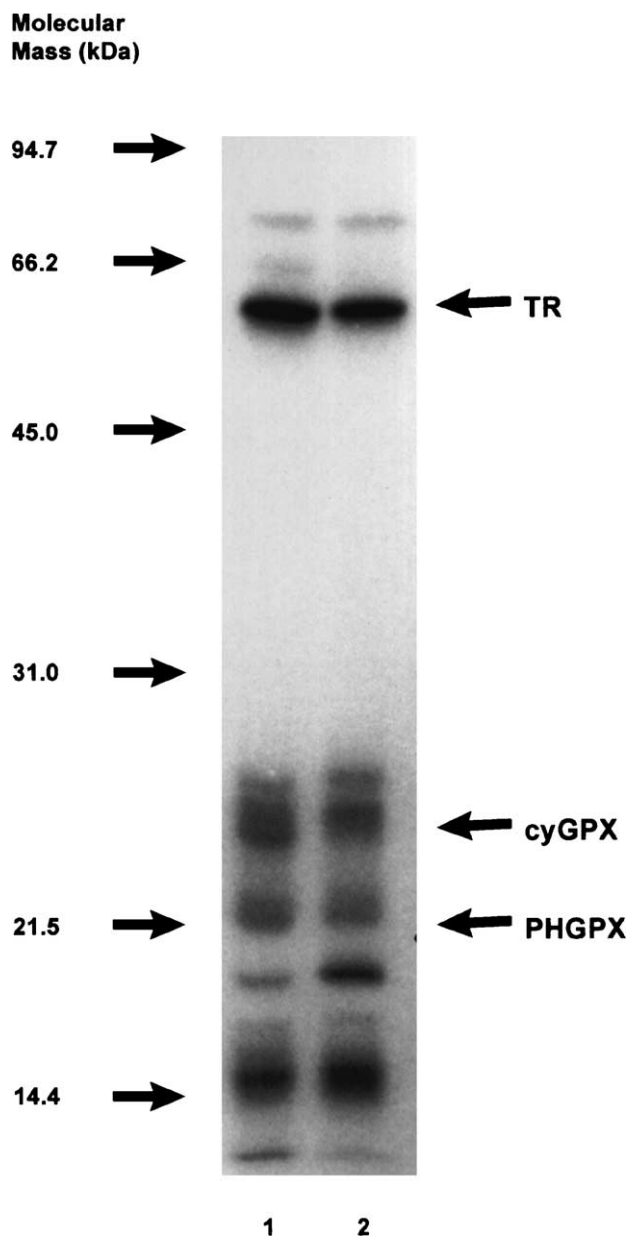


Fig. 4. Autoradiograph of an SDS-PAGE gel of the intracellular selenoproteins of HUVEC and EAhy926 cells labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. Lane 1, HUVEC; lane 2, EAhy926 cells. Both lanes were loaded with 25- $\mu\text{g}$  protein. The selenoprotein bands representing cyGPX, PHGPX and TR are indicated.

The [ $^{75}\text{Se}$ ]-labelling of many selenoproteins in BAEC was significantly less than in HUVEC (Fig. 5, right panel). In particular, the expression of the TR band was much less in BAEC than HUVEC, an observation that was confirmed by activity measurements of TR (Table 1).

### 3.4. Se content of culture medium

The EGM2 used for the culture of HUVEC, HCAEC and HUAEC had a Se content of  $30 \pm 2$  nM, which was not significantly different to the Se content of EGM used for the

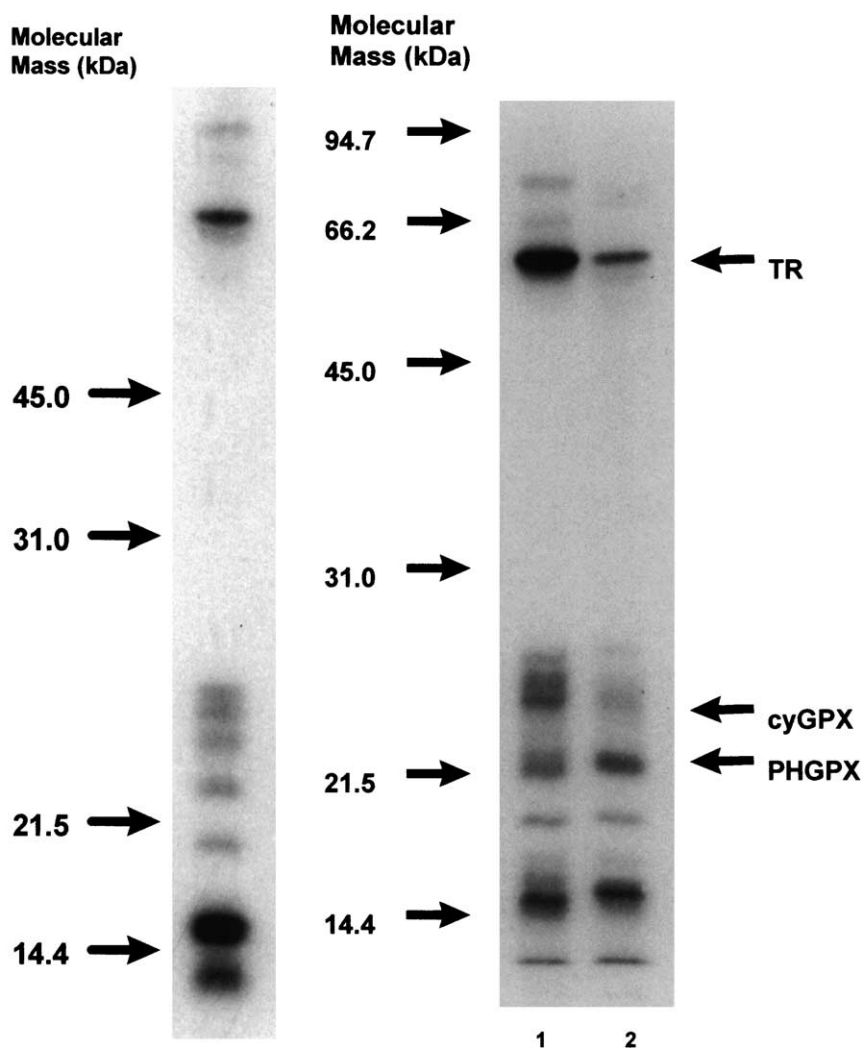


Fig. 5. Left. Autoradiograph of an SDS-PAGE gel of PAEC labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. The lane was loaded with 25  $\mu\text{g}$  of protein. Right. Autoradiograph of an SDS-PAGE gel of the intracellular selenoproteins of HUVEC and BAEC labelled with [ $^{75}\text{Se}$ ]-selenite (0.02 MBq/ml) for 48 h. Lane 1, HUVEC; lane 2, BAEC. Both lanes were loaded with 25- $\mu\text{g}$  protein. The selenoprotein bands representing cyGPX, PHGPX and TR are indicated.

culture of BAEC ( $43 \pm 10$  nM). In both of these culture media, Se is present as selenious acid. The Se content of the DMEM/10% FCS/HAT culture medium used for the culture of EAhy926 cells was  $18 \pm 2$  nM, with the Se provided by the FBS in the form of selenoprotein. The basal M199 medium used to culture PAEC had no detectable Se content, however, since these cells are grown in the presence of 20% FBS, the final concentration of Se in this culture medium was  $36 \pm 2$  nM present as selenoprotein.

#### 4. Discussion

These studies indicate that for HUVEC, an incubation period of at least 48 h must be employed to achieve maximal  $^{75}\text{Se}$  labelling of selenoproteins (Fig. 1, left panel). This contrasts with human thyrocytes where [ $^{75}\text{Se}$ ]-labelling reaches a steady state by 27 h [5]. This difference may be due to the variability in selenoprotein turnover between

tissues and possibly different rates of [ $^{75}\text{Se}$ ]-selenite uptake between cell types.

In primary culture, HUVEC have a limited replication potential, tending to senesce [27] and, in addition, the activities of some enzymes have been reported to vary with successive passages [28]. We observed no differences in the [ $^{75}\text{Se}$ ]-selenoprotein labelling of HUVEC throughout eight passages of the same preparation under identical growth conditions (Fig. 1, right panel), arguing that the expression of selenoproteins in HUVEC remains constant over at least eight passages. In addition, we found that the selenoprotein profile in four individual preparations of HUVEC showed no marked variability between preparations (Fig. 1, centre). We have also confirmed our previous observation that HUVEC show dominant expression of the 58.1-kDa selenoprotein, TR [6]. Two other major [ $^{75}\text{Se}$ ]-selenoproteins expressed by HUVEC, which were labelled to a much lesser extent than TR, had molecular masses of 21.7 and 24.4 kDa and were provisionally identified as cyGPX and PHGPX,

respectively. Since identification was based on co-migration with purified material and by Western blotting, we cannot exclude the possibility that selenoproteins with identical molecular mass co-migrate with these peroxidases on the SDS-PAGE system.

EC show functional differences according to the vascular bed from which they are derived. Arterial EC differ from venous EC in a number of aspects, such as the production of angiotensin-converting enzyme, ability to form prostacyclin [11] and their cell adhesion molecule response to cytokine stimulation [12]. We compared selenoprotein expression in a range of primary human EC cells using similar culture conditions that included keeping the concentration and chemical form of Se in the culture medium constant. The [ $^{75}\text{Se}$ ]-selenoprotein profiles of HUVEC and HCAEC were remarkably similar, with the only observable difference being a slight increased expression of TR in HCAEC that was confirmed by assay of TR concentration. The [ $^{75}\text{Se}$ ]-labelled selenoprotein profiles of EC isolated from the human umbilical vein (which is arterial-like in the umbilical cord) and human umbilical artery of the same umbilical cord were also very similar. The most obvious difference was that HUVEC expressed an additional selenoprotein (27 kDa) when compared to HUAEC (Fig. 2). We did not characterise this selenoprotein but it has a molecular mass similar to type I iodothyronine deiodinase [29].

Distinct differences in the pattern of selenoprotein expression between human EC and EC isolated from bovine and porcine vascular beds were observed. In HUVEC and other human EC studied, TR was the dominantly [ $^{75}\text{Se}$ ]-labelled selenoprotein. In PAEC the expression of TR was relatively weak, the predominant selenoprotein having an approximate molecular mass of 15 kDa (Fig. 5 left panel). This 15-kDa [ $^{75}\text{Se}$ ]-selenoprotein was also found in HUVEC but labelled to a lesser extent. The identity of the 15-kDa selenoprotein was not established, although its electrophoretic mobility is consistent with it being either the uncharacterised 15-kDa selenoprotein described by Gladyshev et al. [30] or the Se-containing protein, epidermal fatty acid-binding protein (E-FABP) [30]. Masouyé et al. [31] have demonstrated the presence of E-FABP in HUVEC but they were unable to detect the protein in the endothelium of the umbilical vein from which the EC were isolated, suggesting that the expression of E-FABP may be induced by cell culture.

The pattern of [ $^{75}\text{Se}$ ]-selenoprotein expression in BAEC also differed considerably from that observed in HUVEC (Fig. 5, right panel). The [ $^{75}\text{Se}$ ]-labelling of the majority of selenoproteins was significantly lower in BAEC compared to HUVEC and TR expression and activity was considerably lower in BAEC than in HUVEC (Table 1). The expression of selenoproteins is modified by the Se status of the cell. However, the Se content and the chemical form of Se (ie selenious acid) present in the culture medium for HUVEC (EGM2) and BAEC (EGM) were identical. This suggests that differing selenoprotein profiles seen in

HUVEC and BAEC is not a consequence of altered Se status.

The selenoprotein profile found for EAhy926 cells was very similar to HUVEC and HCAEC, however, the activity of cyGPX in EAhy926 grown in basal medium was significantly lower than in HUVEC or HCAEC (Table 1). Inclusion of 40 nM selenite into the basal culture medium resulted in significant increases in the expression of each of the selenoenzymes, such that cyGPX activity increased to levels that were significantly higher than that found in HUVEC (Table 1). These data suggest that the small differences in selenoenzyme activity observed between HUVEC and EAhy926 grown in basal medium are due to the limiting supply of Se in the basal medium used to culture EAhy926. This basal medium has Se included as selenoprotein incorporated into the FBS. The Se in extracellular selenoproteins is not readily available to the cell whilst selenite is rapidly taken up by the cell and utilised for selenoprotein synthesis.

This study has firmly established the suitability and consistency of using EC from the human umbilical vein as a model to study the effects of Se on EC function in relation to atheroma development in the coronary artery. EC isolated from pig and bovine aorta showed marked differences in selenoprotein profile when compared to human cells, particularly as regards the expression of the antioxidant enzyme TR. Only small differences were observed in selenoprotein expression and activity in EC originating from the coronary artery and other various human vasculature and the human cell line EAhy926.

We conclude that whilst the most appropriate cell culture model for the study of selenoprotein expression in atherosclerotic disease in man might be HCAEC, the supply of such cells is limited and even then the cells are often isolated from diseased vessels. Our studies suggest that HUVEC and possibly EAhy926 cells are suitable alternative model systems to HCAEC in which the role of selenoproteins in protecting against atheroma formation can be studied.

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